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Insulin increases sodium reabsorption in diluting segment in humans: Evidence for indirect mediation through hypokalemia

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Insulin increases sodium reabsorption in diluting segment in humans: Evidence for indirect mediation through hypokalemia. To examine the mechanism of renal sodium (Na) and potassium (K) retention during insulin infusion, seven healthy volunteers underwent clearance studies without (time control) and with insulin infusion (40 mU bolus, followed by 1 mU/kg/min for 150 min). Maximal free water clearance and fractional lithium clearance (FE_{Li}) were used to analyze renal sodium handling. Insulin decreased Na excretion (from 189 ± 25 to 121 ± 19 μmol/min, $P < 0.01$) and K excretion (from 64 ± 8 to 19 ± 1 μmol/min, $P < 0.01$), but did not change in glomerular filtration rate. FE_{Li} increased from 29.8 ± 1.9 to 32.3 ± 1.9% ($P < 0.05$), minimal urine osmolality decreased from 59 ± 3 to 46 ± 3 mOsm/kg ($P < 0.01$), and the diluting segment reabsorption index increased from 88.0 ± 0.9 to 93.7 ± 0.9%, $P < 0.01$). Insulin also decreased plasma K, from 3.91 ± 0.08 to 3.28 ± 0.08 mmol/liter, $P < 0.01$. In a third clearance study KCl was infused simultaneously (3.75 μmol/kg/min) to prevent this fall in plasma K. In this study insulin had no effect on Na and K excretion and diluting segment reabsorption, but the rise in FE_{Li} remained. In a fourth clearance study NaCl (3.75 μmol/kg/min) instead of KCl was infused together with insulin. This manoever did not prevent the Na and K retaining effect of insulin, nor any of its effects on renal sodium handling parameters. These data suggest that Na and K retention during insulin infusion are largely secondary to hypokalemia, which causes increased reabsorption in the diluting segment.

In 1953 Miller and Bogdonoff [1] reported that administration of insulin in humans causes sodium retention. Later studies in humans have confirmed this effect [2–5]. We found recently that the sodium (and potassium) retaining effect of insulin infusion is abolished when the associated hypokalemia is prevented by simultaneous potassium infusion [6]. This suggests that insulin causes sodium retention in an indirect manner, that is, via hypokalemia, which is surprising since the sodium retention of insulin is generally considered a direct renal effect of this hormone. Indeed, intrarenal insulin infusion decreases sodium excretion in the ipsilateral kidney [7]. Insulin also decreases sodium excretion in the isolated perfused kidney [8]. Insulin has been shown to increase volume reabsorption in microperfused rat proximal tubules [9], to stimulate Na^+/H^+ exchange in cultured rabbit proximal tubule cells [10], and to enhance Na^+,K^+-ATPase activity in cultured toad kidney cells [11].

One can argue, therefore, that our observation that sodium and potassium retention by insulin was prevented by simultaneous infusion of KCl [6] concerns a nonspecific effect of the applied chloride load. To further investigate whether the main sodium retaining mechanism of insulin in vivo indeed concerns the indirect effect of hypokalemia we formulated two hypotheses. First, if this were true, prevention of hypokalemia by KCl infusion should not only be able to abolish the sodium retention of insulin, but also have the opposite effect on tubular sodium handling. Second, infusion of an equivalent amount of chloride with another ion, NaCl instead of KCl, should not prevent the renal effects of insulin. We presently studied these options in humans, analyzing segmental sodium reabsorption during insulin infusion without and with simultaneous KCl or NaCl infusion.

Methods

Studies were carried out in seven healthy males (age 24 ± 3 years). Informed consent was obtained, and the protocol was approved by the Hospital Committee for Studies in Humans. The subjects took a diet containing a fixed amount of sodium (300 mmol) and potassium (80 to 100 mmol) for eight days, and clearance studies were performed on days 4, 5, and 8. Within four months, a fourth clearance study was performed after four days of the same dietary regimen. The diet was provided by the metabolic ward, where the subjects payed a daily visit at noon. Compliance to the diet was assessed by measurement of 24-hour urine electrolyte excretion throughout the study.

Clearance studies were performed in the metabolic ward, after an overnight fast. Before each clearance study a single oral dose of 400 mg lithium carbonate was taken at the prior evening at 2200 hours. Between 0830 and 0930 hours a 20 ml/kg water load was ingested, and additional water matching urinary output was taken throughout the clearance study. The subjects were supine, and at 0900 hours a constant infusion of inulin (polyfructosan, Inutest^R) was started after a priming dose, through an indwelling canula in a lower arm vein. A vein in the contralateral arm was canulated for repeated blood sampling. After a two-hour equilibration period, when urine osmolality had reached a minimal value, seven 30-minute urine collections were made (from 1100 to 1430 hours). Blood samples for clearance measurements were taken halfway through each collection period. Blood for plasma renin activity and aldosterone concentration was taken during the second and final urine collection periods. Throughout the clearance study blood pressure was measured at 10 minutes intervals with an automatic

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sphygmomanometer device (Omega, 1000, Invivo Research Laboratory Inc., Tulsa, Oklahoma, USA).

The first clearance study (day 4) served as a time-control study. During the second clearance study (day 5) insulin was infused (40 mU/kg as a bolus, followed by 1 mU/kg/min constant infusion) after the second urine collection. Glucose was infused as follows: a 5% infusion constantly at 8 ml/kg/hr, and a 10% infusion adjusted individually to maintain euglycemia. During the third clearance study (day 8) the same infusion regimen was applied as on day 5; however, potassium chloride was added to the 5% glucose infusion (28 mmol/liter) to obtain an infusion rate of 3.75 μmol/kg/min. This infusion rate effectively prevented hypokalemia during insulin infusion in a study by DeFronzo et al [12]. The fourth clearance study was similar as the third, except that now NaCl was infused instead of KCl (equivalent amount).

Plasma glucose was assessed by an instant hemoglucotest with a reflectance photometer during the clearance studies, and reassessed later by spectrophotometry. Blood and urine samples were analyzed for sodium and potassium (flame photometry), chloride (Technicon RA-1000 autoanalyzer), lithium (Perkin Elmer 3030 Atomic Absorption Spectrophotometer), osmolality (Advanced Osmometer), and inulin. The latter was determined photometrically with indolacetic acid after hydrolyzation to fructose [13]. Plasma renin activity (fmol Angiotensin I/liter/sec) and aldosterone (pmol/liter) were measured by radioimmunoassay.

Calculations and statistical analysis

Clearances and fractional clearances were calculated by standard formula. Inulin clearance (C_{inulin}) was taken as marker for glomerular filtration rate (GFR). Free water clearance (C_{H_2O}) was regarded as index of sodium reabsorption in the diluting segment, defined as the nephron beyond the point of isotonicity in the thick ascending limb of Henle's loop (14). C_{H_2O} plus chloride clearance ($C_{H_2O} + C_{Cl}$) was regarded as an index of solute delivery to the diluting segment. The term $[(C_{H_2O} + C_{Cl})/C_{inulin}]$ therefore represents an approximation of fractional solute delivery to diluting segment, and the term $[C_{H_2O}/(C_{H_2O} + C_{Cl})]$ an approximation of diluting segment reabsorption. The validity of these terms and possible pitfalls have been discussed by others [15]. The lithium clearance has been advanced as an index of volume delivery to the distal tubules [16], but this method also has its limitations [17]. Therefore, appropriate caution was applied in the interpretation of these clearance data.

Statistical analysis was performed by one-way analysis of variance for repeated measures. Statistical significance of differences was tested by Student's *t*-test for paired observations using Bonferroni's protection. Values are presented as means ± standard error.

Results

During the study there were no significant changes in body weight (73.7 ± 2.4 and 73.7 ± 2.2 kg on days 3 and 7, respectively) and 24-hour urine sodium excretion (315 ± 10 and 295 ± 26 mmol on days 3 and 7). Prior to the final clearance study body weight was 74.7 ± 2.0 kg, and 24-hour urine sodium excretion 290 ± 24 mmol.

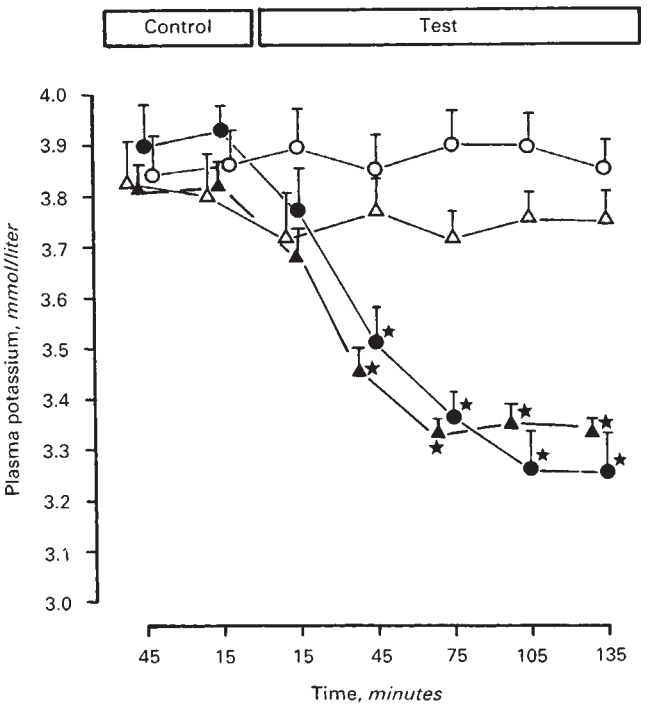

Fig. 1. *Plasma potassium during time-control study (○), insulin infusion (●), insulin + KCl infusion (△), and insulin + NaCl infusion (▲).* "Test" denotes the infusion period. Significant differences from time-control study are indicated by *, $P < 0.01$.

Time control study

Changes in plasma potassium, and urinary sodium and potassium excretion are depicted in Figures 1 and 2. There was no significant change in these variables during the time control study. For the analysis of the renal sodium handling data we compared means of the two baseline collection periods with means of the final two test periods (Tables 1 and 2). There were no significant changes, except for a decrease in maximal urine flow, whereas urine osmolality was maintained at a minimal level. Plasma renin activity decreased ($P < 0.05$), whereas plasma aldosterone, blood pressure and plasma glucose were not significantly different in baseline and test periods (Table 3).

Insulin infusion study

In comparison to the time control test, insulin infusion clearly decreased plasma potassium (Fig. 1), and urinary sodium and potassium output (Fig. 2). Stable low levels were obtained in the two final collection periods, which therefore were used to analyze changes in renal sodium handling (Table 1). Insulin had no effect on GFR, and the fall in sodium and potassium excretion was associated with a fall in fractional excretion of these electrolytes. Fractional chloride excretion decreased as well. In contrast with the time control study, there was a large decrease in minimal urine osmolality, but no decrease in maximal urine flow. The distal delivery term $[(C_{H_2O} + C_{Cl})/C_{inulin}]$ did not change, but fractional lithium excretion increased. The diluting segment reabsorption increased as well. No significant change occurred in plasma renin activity, aldosterone and blood pressure (Table 3). Plasma glucose was clamped effectively between 4 and 6 mmol/liter, as is illustrated

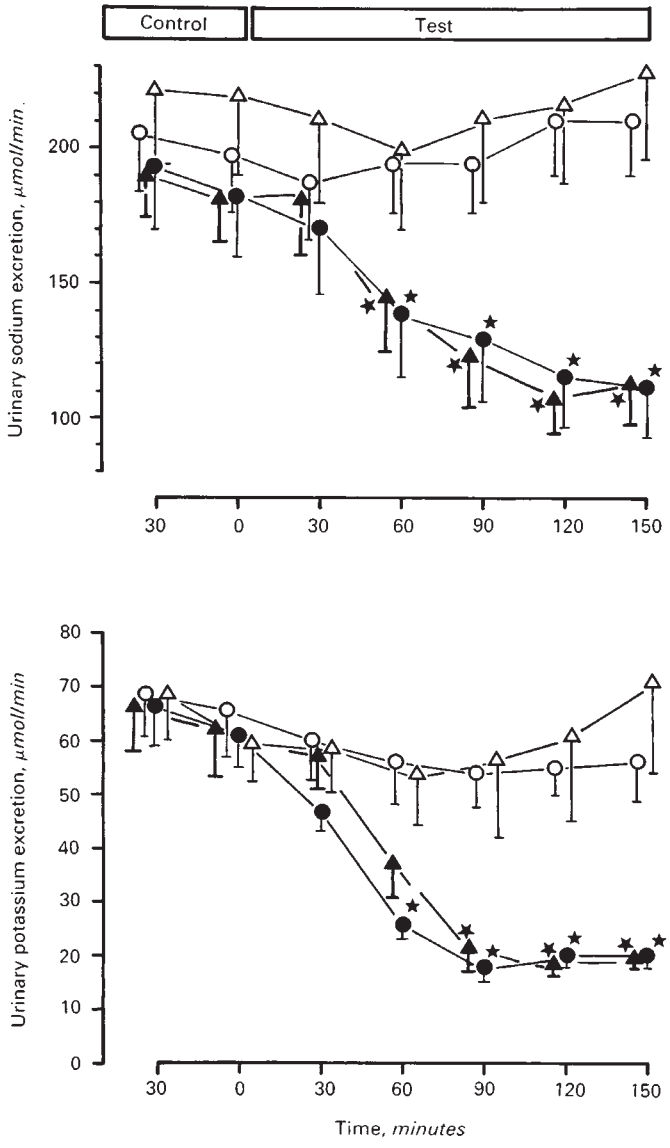

Fig. 2. *Urinary sodium and potassium excretions during time-control study (○), insulin infusion (●), insulin + KCl infusion (△), and insulin + NaCl infusion (▲).* "Test" denotes the infusion period. Significant differences from time-control study are indicated by *, $P < 0.01$.

by the baseline and test period values given in Table 3. The amount of glucose infused averaged 0.75 ± 0.10 g/kg/hr.

Insulin + KCl infusion study

The potassium infusion prevented the decrease in plasma potassium observed during insulin alone (Fig. 1), and also prevented the decrease in urinary potassium and sodium excretion (Fig. 2). Potassium infusion also prevented a number of the changes in renal sodium handling seen during insulin alone: there was no decrease in minimal urine osmolality, and no increase in diluting segment reabsorption (Table 2). However, during this combined insulin + potassium infusion there still was an increase in fractional lithium excretion, and no fall in maximal urine flow as was found in the time control study. Again no significant change occurred in plasma renin activity, aldosterone, and blood pressure (Table 3). In this experiment plasma glucose was also clamped effectively between 4 and 6 mmol/liter (mean values in baseline and test periods are given in Table 3). The amount of glucose needed for this purpose (0.77 ± 0.08 g/kg/hr) was comparable to that infused during insulin alone.

Insulin + NaCl infusion study

In this experiment, instead of KCl, an equivalent amount of NaCl was infused together with insulin and glucose. The decrease in plasma potassium was comparable to that found during insulin alone (Fig. 1). Clearly, although the amount of chloride infused was identical as in the KCl experiment, the NaCl infusion did not prevent the fall in urinary sodium and potassium (Fig. 2) and in urinary chloride excretion (Table 2). The changes in renal sodium handling parameters were also comparable to those observed during insulin alone: a decrease in minimal urine osmolality, an increase in diluting segment reabsorption term and in fractional lithium clearance, and no change in maximal urine flow. There were no significant changes in plasma renin activity, aldosterone, and blood pressure (Table 3). Plasma glucose was again successfully kept at steady values (mean values of baseline and test periods are given in Table 3). The glucose infusion rate (0.80 ± 0.07 g/kg/hr) did not differ from that in the two other insulin infusion experiments.

Discussion

In this study we confirmed our previous finding [6] that sodium and potassium retention by insulin infusion can be prevented by simultaneous infusion of KCl in an amount sufficient to prevent hypokalemia. To find out whether this concerns the non-specific effect of the chloride load, or whether the potassium ion is crucial, we also repeated this experiment during infusion of an identical amount of NaCl. Since this manoever neither prevented the insulin-induced sodium and potassium retention, nor the insulin-induced changes in renal sodium handling parameters (see below), it is likely that hypokalemia somehow plays a mediating role in the sodium retaining effect of insulin. This option was tested also by studying whether KCl could prevent the effects of insulin on renal sodium handling.

Insulin had no effect on GFR, but increased sodium reabsorption. Maximal urine flow and distal delivery term [(C_{H_2O} + C_{Cl})/GFR] did not change. In fact, maximal urine flow remained elevated compared to the time-control study. These data, comparable to observations made by others [1, 3], do not suggest increased sodium reabsorption in the proximal tubules. This contrasts with some data from animal studies. Insulin has been shown to increase volume reabsorption in isolated perfused rabbit proximal tubules [9], and to stimulate Na^+/H^+ exchange and Na-glucose cotransport in proximal tubule brush border membrane vesicles [18, 19] and cultured proximal tubule cells [10]. However, these studies do not discuss the quantitative importance of these effects in vivo. In this respect, micropuncture studies are relevant. With this technique applied in dogs, it was found that insulin reduced proximal tubular sodium reabsorption [7]. Since fractional sodium excretion in the urine decreased as well, it was concluded that insulin increased

Table 1. Plasma potassium and clearance data during time control and insulin infusion experiments

	Time control		Insulin		
	Baseline	Test	Baseline	Test	*P* value
Plasma potassium *mmol/liter*	3.85 ± 0.08	3.88 ± 0.07	3.91 ± 0.08	3.28 ± 0.08[b]	[b]
C_{inulin} *ml/min*	115 ± 4	112 ± 3	115 ± 5	119 ± 6	NS
Urine flow *ml/min*	15.9 ± 1.4	13.9 ± 1.1[a]	15.3 ± 1.3	15.1 ± 1.3	[a]
Urine osmolality *mOsm/kg*	61 ± 3	63 ± 3	59 ± 3	46 ± 3[b]	[b]
C_{H_2O}/C_{inulin} %	10.5 ± 0.9	9.4 ± 0.7	10.5 ± 0.8	10.3 ± 0.8	NS
$(C_{H_2O} + C_{Cl})/C_{inulin}$ %	12.1 ± 1.0	10.8 ± 0.8	12.0 ± 1.1	11.1 ± 0.9	NS
$C_{H_2O}/(C_{H_2O} + C_{Cl})$ %	87.4 ± 1.1	87.2 ± 0.6	88.0 ± 0.9	93.7 ± 0.9[b]	[b]
Sodium excretion *μmol/min*	202 ± 21	206 ± 20	189 ± 25	121 ± 19[b]	[b]
Potassium excretion *μmol/min*	66 ± 10	56 ± 5	64 ± 8	19 ± 1[b]	[b]
Chloride excretion *μmol/min*	117 ± 25	161 ± 17	176 ± 27	94 ± 19[b]	[b]
FE sodium %	1.25 ± 0.11	1.33 ± 0.11	1.20 ± 0.15	0.72 ± 0.11[b]	[b]
FE potassium %	14.9 ± 1.8	12.8 ± 1.1	14.2 ± 1.7	4.8 ± 0.3[b]	[b]
FE lithium %	30.4 ± 2.1	28.4 ± 1.7	29.8 ± 1.9	32.3 ± 1.9[a]	[b]

Values are means (± SEM) of the two baseline periods ("baseline") and the final two test periods ("test"). Significant differences between test and baseline are indicated in the "test" columns by [a] $P < 0.05$, and [b] $P < 0.01$. The left hand column denotes significant differences between the changes during the time control study versus the changes during the insulin infusion study.

Abbreviations are: C, clearance; FE, fractional excretion.

Table 2. Plasma potassium and clearance data during insulin + KCl infusion and insulin + NaCl infusion experiments

	Insulin + KCl		Insulin + NaCl		*P* value		
	Baseline	Test	Baseline	Test	A	B	C
Plasma potassium *mmol/liter*	3.81 ± 0.09	3.74 ± 0.06	3.82 ± 0.08	3.32 ± 0.05[b]	NS	[b]	[b]
C_{inulin} *ml/min*	117 ± 6	122 ± 5	111 ± 5	114 ± 4	NS	NS	NS
Urine flow *ml/min*	15.8 ± 1.5	16.5 ± 1.5	15.3 ± 1.0	15.2 ± 1.1	[a]	NS	[a]
Urine osmolality *mOsm/kg*	62 ± 4	58 ± 3	63 ± 3	51 ± 3[b]	NS	[a]	[a]
C_{H_2O}/C_{inulin} %	10.5 ± 0.8	10.8 ± 0.7	10.6 ± 0.7	10.8 ± 0.7	NS	NS	NS
$(C_{H_2O} + C_{Cl})/C_{inulin}$ %	12.1 ± 1.1	12.5 ± 1.0	12.1 ± 0.9	11.7 ± 0.7	NS	NS	NS
$C_{H_2O}/(C_{H_2O} + C_{Cl})$ %	87.6 ± 1.4	87.2 ± 1.9	88.0 ± 0.7	93.2 ± 0.8[b]	NS	[b]	[b]
Sodium excretion *μmol/min*	221 ± 30	219 ± 32	185 ± 20	112 ± 17[b]	NS	[b]	[b]
Potassium excretion *μmol/min*	64 ± 10	64 ± 17	64 ± 10	20 ± 3[b]	NS	[b]	[b]
Chloride excretion *μmol/min*	193 ± 37	191 ± 38	166 ± 19	95 ± 16[b]	NS	[b]	[b]
FE_{Na} %	1.38 ± 0.20	1.32 ± 0.22	1.23 ± 0.13	0.71 ± 0.11[b]	NS	[b]	[b]
FE_K %	13.9 ± 1.9	14.1 ± 3.1	14.8 ± 2.3	5.2 ± 0.7[b]	NS	[a]	[b]
FE_{Li} %	30.4 ± 1.8	32.7 ± 1.7[a]	33.1 ± 2.0	36.2 ± 1.5[b]	NS	NS	[b]

Values are means (± SEM) of the two baseline periods ("baseline") and the two final test periods ("test"). Significant differences between test and baseline are indicated in the "test" columns by [a] $P < 0.05$, and [b] $P < 0.01$. The right hand columns (A, B, and C) denote significant differences between the tests: (A) changes during the time control versus changes during the insulin + KCl infusion study; (B) changes during insulin infusion alone versus changes during the insulin + KCl infusion study; (C) changes during the time control study versus changes during the insulin + NaCl infusion study. There were no significant differences between the changes during insulin alone and the changes during the insulin + NaCl infusion study.

Abbreviations are: C, clearance; FE, fractional excretion.

Table 3. Plasma glucose, renin activity, and aldosterone, and blood pressure during each experiment

	Time control		Insulin		Insulin + KCl		Insulin + NaCl	
	Baseline	Test	Baseline	Test	Baseline	Test	Baseline	Test
Plasma glucose *mmol/liter*	4.3 ± 0.1	4.2 ± 0.2	4.5 ± 0.2	4.2 ± 0.2	4.3 ± 0.1	4.4 ± 0.3	4.0 ± 0.3	4.1 ± 0.3
Plasma renin activity *fmol/liter/sec*	153 ± 36	117 ± 26[a]	180 ± 65	195 ± 74	191 ± 54	186 ± 51	145 ± 36	171 ± 40
Plasma aldosterone *pmol/liter*	214 ± 33	183 ± 19	221 ± 31	196 ± 35	204 ± 20	264 ± 47	160 ± 20	127 ± 18
Systolic blood pressure *mm Hg*	125 ± 3	127 ± 4	124 ± 3	128 ± 3	124 ± 3	126 ± 2	127 ± 1	129 ± 3
Diastolic blood pressure *mm Hg*	72 ± 2	70 ± 2	73 ± 2	71 ± 2	74 ± 1	70 ± 2	70 ± 2	67 ± 2

Values are means (± SEM) of the baseline collection periods ("baseline") and of the two final test collection periods ("test").

[a] Significant differences between test and baseline, $P < 0.05$.

reabsorption somewhere beyond the proximal tubules. The present data in humans accord well with this idea.

The decrease in minimal urine osmolality and increase in the term $[C_{H_2O}/(C_{H_2O} + C_{Cl})]$ suggest that insulin stimulated sodium reabsorption in the diluting segment. Kirchner [20] found no effect of insulin on chloride reabsorption in micropuncture-accessible proximal tubules. However, marked stimulation of chloride reabsorption in Henle's loop appeared likely in view of the decrease in early distal chloride delivery. Since early distal fluid delivery was not decreased, it was suggested that insulin increased chloride reabsorption in the thick ascending limb of Henle's loop [20]. Thus, the presently observed fall in sodium

and potassium excretion may reflect increased re-absorption in Henle's loop.

Under the conditions of our study, the diluting segment also comprises the nephron segments downstream from Henle's loop [14, 15]. In this respect, it is relevant that Kirchner [20] did not find an effect of insulin on chloride reabsorption in micropuncture-accessible distal convoluted tubules. Since insulin enhances aldosterone-stimulated sodium reabsorption and potassium secretion, and Na^+,K^+-ATPase activity in cultered toad urinary bladder and toad kidney cells [11, 21], it is possible that insulin increased sodium reabsorption in the collecting tubules. However, the simultaneous decrease in potassium excretion speaks against this option, since increased sodium reabsorption at this site would favor potassium secretion [21, 22]. Instead, it seems likely that insulin impaired potassium secretion in this segment by a combination of decreased sodium delivery and peritubular potassium concentration, both important determinants of potassium secretion in the collecting tubules [22, 23].

Infusion of KCl fully prevented the fall in urine osmolality and rise in diluting segment reabsorption caused by insulin, also in agreement with the notion that hypokalemia plays a mediating role in the sodium retaining effect of insulin. In view of the above considerations, increased sodium reabsorption in Henle's loop during insulin infusion seems a conceivable option, but we are not aware of any direct evidence that acute hypokalemia increases NaCl reabsorption in this segment. However, that this also is a conceivable option can be derived from the opposite observation during acute hyperkalemia. Milanes and Jamison [24] found increased early-distal sodium and potassium delivery after a potassium load in partially nephrectomized rats, although this effect did not exceed the effect of a corresponding NaCl load. Kirchner found decreased chloride reabsorption in Henle's loop after a potassium load in micropuncture-studied rats [25]. Stokes [26] demonstrated in in-vivo microperfusion experiments that an increase in peritubular potassium concentration reduced NaCl reabsorption and changed potassium reabsorption into secretion in medullary thick ascending limbs. In view of these observations during acute *hyper*kalemia, it seems not unreasonable to assume that acute *hypo*kalemia would increase the reabsorption of sodium, potassium, and chloride in Henle's loop, nor that prevention of hypokalemia would prevent this effect. An increase in peritubular potassium concentration was shown to increase sodium reabsorption in late distal tubule and the cortical collecting tubule in rodents [23, 27]. This argues against these segments as sites where hypokalemia might mediate the sodium retaining effect of insulin.

Insulin infusion slightly increased lithium excretion. Increased lithium clearance together with sodium retention has been found earlier during glucose infusion [28], a situation in which insulin is also high. This finding confirms that insulin did not increase sodium reabsorption in the proximal tubules. However, although most lithium is undoubtedly reabsorbed in the proximal tubules, there is evidence that some is reabsorbed in Henle's loop [17, 29]. Even though this may not be much, one would not expect a rise in lithium excretion if sodium reabsorption in Henle's loop is increased. Therefore, we cannot exclude that insulin indeed increases sodium reabsorption somewhere distal from Henle's loop. In any case, it is likely that the increase in sodium reabsorption occurred independently of site and mechanism of decreased lithium reabsorption, since sodium and lithium are supposed to be transported at least in the same direction in those segents where both are handled [17]. Therefore, the finding that, by exception, KCl did not prevent this effect of insulin, is not in disagreement with the idea that KCl infusion interfered with the mechanism by which insulin causes sodium retention.

In the time control study the PRA decreased, probably reflecting diurnal rhythm. This was not seen during insulin infusion, suggesting that insulin stimulates renin release, in accordance with other recent data in humans by Trovati et al [30]. The macula densa mechanism might be involved, assuming that insulin indeed increases sodium reabsorption upstream. In that case we would expect that KCl would have prevented the renin stimulation, which was not found. However, it should be mentioned that KCl did prevent insulin-induced renin stimulation in Trovati et al's study [30]. Whether this discrepancy between the two studies concerns a methodological problem is not apparent. That the potassium ion may be involved also in this effect of insulin is suggested by the observation in experimental animals that administration of potassium, independent of the accompanying anion, suppresses plasma renin [31, 32]. In neither of the present experiments significant were changes in aldosterone found, despite important variations in plasma potassium. Perhaps this is due to the high sodium intake in our study, since potassium-related aldosterone variability is probably low at low levels of angiotensin II [33]. Anyway, it is unlikely that aldosterone mediated the decrease in urinary sodium and potassium excretion during insulin infusion, nor prevented this change during the combined infusion.

Intrarenal insulin infusion has been shown to increase ipsilateral sodium reabsorption [7], clearly in favor of direct mediation of this effect. The presence of high concentrations of insulin receptors in the kidney, in particular along the thick ascending limbs of Henle's loop [34], is compatible with a direct effect. However, many of these receptors may be involved with insulin degradation instead of the mediation of transport effects [35], and it is unknown whether a direct effect is indeed the main mechanism by which insulin causes sodium retention in vivo. Our data suggest a crucial role for hypokalemia: insulin either increases sodium reabsorption through mediation of hypokalemia, or exerts this effect directly provided that plasma potassium is reduced. However, clearance techniques have obvious limitations, and from the present studies in humans it is impossible to exactly define the site of interaction of potassium and insulin in the kidney, or even to make certain whether such interaction exists. Also, we cannot discern whether the proposed effect of potassium concerns a direct action of potassium on tubule cells, or requires further intermediate steps, such as changes in renal vascular tone. Animal studies will be needed to resolve these questions.

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